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RESEARCH PAPER

Effects of Agricultural Carbon Sources On Water Quality and Phytoplankton Community Composition in Flocponic System

Kenneth Rono[1*], Geraldine Matolla[2], Julius Otieno Manyala[3], Frank Onderi Masese[4]

[1,2,4]Department of Fisheries and Aquatic Science , School of Environmental Sciences, University of Eldoret , Eldoret, P.O BOX 1125-30100, Kenya*
[3]Department of Natural resource, School of Special Planning and Natural Resource Management, Jaramogi Oginga Odinga University of Science and Technology, Bondo, P.O BOX 210-40601, Kenya

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Corresponding Author

Kenneth Rono

E-mail: kennethrono01@gmail.com
Tel: +254700710865

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Abstract

Carbon products promote aggregate floc-rich plankton, with diverse roles in flocponic production. Availability, low-cost, and chemical composition of agricultural by-products make them ideal substrates for phytoplankton production. Phytoplankton maintains water quality by reducing toxic substances, but it is problematic under some conditions. Therefore, the study evaluates how agricultural carbon sources affect flocponic phytoplankton community composition and water quality. Five treatments (wheat-bran, Rhodes-hay, maize-cob, maize-stables, and lucerne-hay) and a control (no by-product) were employed in a complete randomized design, each in triplicate for nine weeks. Each treatment and control had Nile tilapia (0.155 ± 0.01 g) and rice (seeds) densities of 98 m^{-3} and 250 m^{-2}, respectively. Temperature, pH, dissolved oxygen, and salinity levels did not differ significantly between treatments and control. However, TDS, soluble reactive phosphorus (SRP), ammonia, nitrite, and nitrate showed significant differences ($p<0.05$) between treatments and control. Lucerne-hay exhibited the highest nitrate levels (0.9 ± 0.06 mg L^{-1}), SRP (0.6 ± 0.05 mg L^{-1}), and the lowest ammonia and nitrite levels compared to other treatments and control. Lucerne-hay had the highest phytoplankton diversity (2.48), while the control (1.37) had the least. Further, there were significant differences in phytoplankton abundance, with lucerne-hay having the highest Charophyta (1.45 ± 0.02 indsL^{-1}), Chlorophyta (1.60 ± 0.02 indsL^{-1}), and Ochrophyta (1.64 ± 0.03 indsL^{-1}) abundance, while the control had the least. The result of the study revealed that carbon sources influence flocponic water quality and phytoplankton. The composition and solubility of lucerne-hay and wheat-bran may have improved water quality and phytoplankton. The study suggests that lucerne-hay and wheat-bran are the best flocponic carbon sources for phytoplankton and water quality.

Introduction

The global demand for safe and healthy food continues to rise in response to the growing human population, which is expected to reach 9.7 billion people by 2050 (UN, 2019). The demand for freshwater fish is increasing due to rising food demand, economic development, shifting consumption patterns, and animal protein competition for human and livestock food (Strauch *et al.*, 2019; Pruter *et al*., 2020). Freshwater fish's competitiveness has directly influenced fish farming, intensifying Nile tilapia and catfish (Strauch *et al*., 2019; Pruter *et al.*, 2020). In that case, intensive aquaculture systems are increasing, though organic and inorganic wastes adversely affect the environment (Cao *et al*., 2007; Farmaki *et al*., 2014). Hence, investment and research in sustainable food production technologies are essential to produce enough food while minimizing resource use and environmental impacts (Pretty *et al*., 2010; Boyd *et al*., 2020).

Most aquaculture production globally is either intensively farmed in cages or semi-intensively raised in pond systems (FAO, 2020). Ponds and cages are efficient for producing fish when properly managed and require little investment in technology (Masser, 2012; Tucker, 2012). However, poor management, such as untreated effluents or disregarding the environment's carrying capacity, may lead to environmental pollution and outbreaks of fish diseases (Boyd *et al*., 2020; Henares *et al*., 2020). Therefore, efficient aquaculture systems such as recirculating, aquaponic, biofloc, and flow-through fish farming can contribute sustainably to fish production for a healthy human diet (Thilsted *et al*., 2016; FAO, 2020). However, flow-through systems require a large amount of water compared to recirculating and aquaponic systems that recycle water, even though they are more expensive to operate (Forster & Slaski, 2010; Engle *et al*., 2020). Closed aquaculture systems have attracted interest for further research due to their low water consumption and waste output (Soaudy *et al*., 2018; Khanjani and Sharifinia, 2020; Pinho *et al*., 2021). Biofloc technology is one of these systems; it works with the idea of a microbial loop and helps certain types of microbes grow. For example, it supports the growth of plankton, heterotrophic, and nitrifying bacteria. Shrimp and some fish eat these bacteria (Avnimelech, 2015; Emerenciano *et al*., 2017; Samocha, 2019; Boyd *et al*., 2020). However, these systems experience high nitrate and phosphorus buildup, rely heavily on electricity for proper operation, and operate as monocultures that do not effectively utilize waste products (Badiola *et al*., 2018; Walker *et al*., 2020).

Flocponics is a strategy for circular food production that enhances water quality by combining biofloc-based aquaculture with hydroponics (Pinho *et al*., 2021). Combining hydroponic systems (soilless plant gardening) with biofloc systems is a cost-effective and environmentally friendly technology that simulates a natural ecosystem (Boyd *et al*., 2020). Reusing nutrients to create circular food minimizes environmental effects while increasing food production and cutting costs associated with fertilizer and water (Bohnes *et al*., 2019; Reid *et al*., 2020). The idea is to increase food security by recycling nutrients from fish waste (Kuhn *et al*., 2010; Pinho *et al*., 2021). Various microorganisms, including fungi, bacteria, microalgae, protozoans, and rotifers, collaborate to form flocs from organic waste (Avnimelech, 2009). The floc contains around 30 to 40% organic materials, such as colloids, organic polymers, and dead cells, which other organisms can use and reintegrate into the productive chains (Avnimelech, 2009). Specifically, planktons are the primary micro- and macroscopic organisms that produce an initial chain of food webs and indicators of water quality (Nuraina *et al*., 2020). Planktons in the biofloc system provide nutrients such as proteins, amino acids, and fatty acids to cultured species, as well as remove surplus nutrients (Wasielsky *et al.*, 2006; Azim & Little, 2008; Emerenciano *et al*., 2012; Emerenciano *et al*., 2013; Emerenciano *et al*., 2017). For flocponic technology to work, creating and maintaining diverse floc aggregates with carbon sources that drive floc condition and maintain system integrity is important (Soedibya *et al*., 2022). It is, therefore, critical to know the available and best carbon sources that stimulate and improve phytoplankton growth and diversity since plankton (phytoplankton and zooplankton) are fish nutrients and biological water quality

indicators in aquaculture (Castro-Mejía *et al.*, 2017). Flocponics necessitate using a carbon source with suitable carbon-to-nitrogen ratios (C: N ranging from 10 to 20:1) (Pinho *et al.*, 2021).

One of the main factors affecting floc characteristics is the carbon source, which usually differs in carbon and nutrient (N and P) content and degradability (El-Sayed, 2021). For this reason, carbon sources are beneficial when they facilitate quick nutrient removal and large-volume production of floc (Khanjani & Sharifinia, 2020). Different carbon sources such as acetate, corn, starch, glycerol, molasses, rice bran, molasses, glucose, and sucrose have been the drivers for the development of biofloc for fish, prawns, shrimps, and crayfish (Dauda, 2019). Some studies have checked the effects of various carbon sources and found out which ones are best for fish and crustaceans in biofloc systems (Ahmad *et al.*, 2016; Rajkumar *et al.*,2016; Dauda *et al.*,2017; Khanjani *et al.*, 2017; Bakhshi *et al.*, 2018). Nevertheless, there is no information available on the effects of different carbon sources on the flocponic production of Nile tilapia, rice, and plankton. Furthermore, no studies have researched organic carbon sources such as lucerne-hay, Rhodes-hay, maize-stable, maize-cob, and wheat-bran in flocponic systems or biofloc technology. Such materials will reduce the core competition of refined organic and inorganic carbon sources and promote aquaculture growth with little or no effluent to the environment. Hence, there is a need to establish flocponic systems using inexpensive and commonly available carbon sources. The application of these products in flocponics is promising due to their composition, cost, and availability. Therefore, the study evaluates how agricultural carbon sources affect flocponic phytoplankton community composition and water quality.

Materials and Methods

Study Area

The study was conducted at the University of Eldoret fish (UoE) hatchery for 63 days from May 2022 to November 2022 under greenhouse conditions and temperatures ranging from 26 to 30ºC. The campus is 9 Km Northeast of Eldoret Municipality on the Eldoret-Ziwa Road. The University of Eldoret is within Rift Valley Province, Uasin Gishu County, and Eldoret Town (Kenya).

Experimental Design

The experiment set up included 18 rectangular indoor plastic fish tanks (1.3 m by 1 m by 1 m in length, width, and depth, respectively) using a flocponic system. Nile tilapia fry with similar mean weight (0.16 ± 0.01 g) and length (2.16 ± 0.03 cm) were randomly selected and stocked at the same density (98 fry m^{-3}) in each system. Rice seeds with the same density of 250 plants (seeds) m^{-2} were planted in a suspended plastic egg tray of 100 cm by 30 cm in a flocponic fish-holding unit. Gravels of 0.5 inches were added into the trays to hold and act as the substrate for the rice seeds' germination and growth. The treatments were wheat-bran, Rhodes-hay, maize-cobs, maize-stables, lucerne-hay agricultural by-products, and control (no products), respectively (Figure 1). The treatments were in triplicates in a completely randomized design. Stoichiometry analysis was conducted to calculate each carbon source's carbon, nitrogen, and phosphorus (C: N: P) ratios and quantities. The experimental research used rice seeds from the Ahero rice scheme agro-vet Kisumu County. University of Eldoret (UoE) fish hatchery provided the male sex-reversed *O. niloticus* fingerlings for the research experiment. We purchased commercial fish diets with the same crude protein (30%) from Kenya Marine and Fisheries Training Institute Sangoro and administered to fish in all the treatments. Fish were fed thrice daily, at 0930, 1230, and 1630 h.

Proximate analysis of organic carbon sources

All ground wheat-bran, Rhodes-hay, maize-cob, maize-stables, and lucerne-hay proximate analyses were determined in triplicate, according to standard AOAC methods (AOAC, 1998). Samples were dried in an oven at 60°C until constant weight to determine moisture content. Ash was determined by a combustion method at 550°C for four hours, while crude protein was measured by nitrogen analysis (N x 6.25) using the Kjeldahl method. The crude fiber was determined by digesting dried lipid-free residue with 1.25% sulfuric acid and 1.25% sodium hydroxide and calcining it. We analyzed crude lipid analysis using an automatic fat extraction

system (SOCS PLUS-SCS 08 AS, Pelican Equipment, Chennai, Tamil Nadu, India). We finally analyzed carbon and nitrogen using the colorimetric determination method and phosphorus by persulfate digestion followed by acid-molybdate determination (Duguma *et al*., 2014) (Table 1).

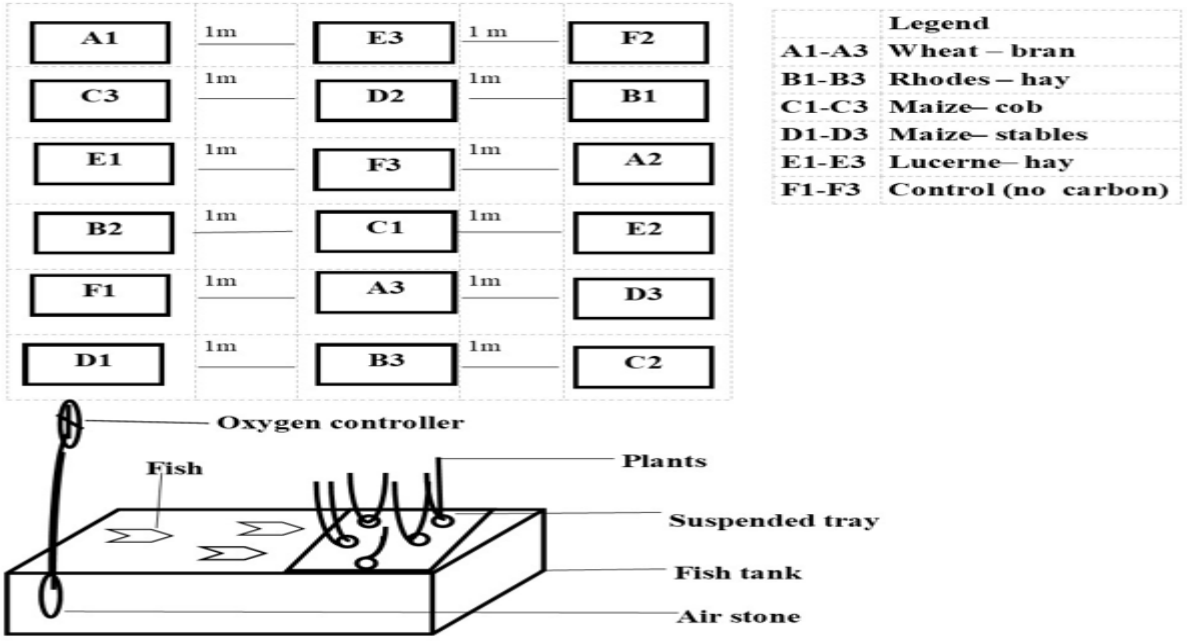

Figure 1. Experimental treatments (wheat-hay, Rhodes-hay, maize-cob, maize-stables, and lucerne-hay) and control layout design in a flocponic set-up.

Table 1. Proximate analysis of organic carbon sources (the daily amount of carbon source addition calculation: using a 15:1 carbon-to-nitrogen ratio)

	Treatments				
Parameters (% in 1g)	**Wheat-bran)**	**Rhode-hay**	**Maize-cob**	**Maize-stable**	**Lucerne-hay**
Ash (%)	5.20± 0.05	7.05±0.10	2.91±0.05	3.30±0.05	7.55± 0.00
Carbon (%)	22.08±0.12	21.18±0.06	23.72±0.12	23.06±0.06	30.01± 0.12
Nitrogen (%)	2.074±0.03	1.41±0.02	1.61±0.01	1.48±0.00	3.41± 0.01
Phosphorus (%)	0.51±0.00	0.43±0.00	0.34±0.27	0.06±0.00	1.1±0.00
Protein (%)	12.96± 0.15	8.8±0.10	10.06±0.04	9.25±0.02	21.3± 0.03
C:P per (1g)	43.3:1	49.3:1	69.8:1	384.3:1	27.3:1
C: N per (1g)	10.7:1	15:1	14.7:1	15.6:1	8.8:1
C: N:P per (1g)	40.9:3:1	49.3:3:1	69.8:5:1	384.3:25:1	27.3:3:1
Quantity (g) in 15:1 (C: N) daily addition to flocponic system	1.36	1.00	1.02	0.96	1.67

Flocponic inoculation

In a flocponic experiment, inoculation was carried out using a similar 15:1 carbon-to-nitrogen ratio of ground wheat-bran, Rhodes-hay, maize-cob, maize-stables, and lucerne-hay. Initial inoculation was employed for one month to enable microbial community stimulation before stocking Nile tilapia and rice. The carbon sources were measured daily, mixed with 100 ml of water, and left overnight in an anaerobic environment before being applied to each flocponic set treatment daily to improve texture for faster breakdown by bacteria (De Schryver *et al*., 2008). Inoculation was done before and continuously after stocking to provide the system with a substrate and bacterial growth (Crab *et al*., 2012). Continuous artificial aeration was used to achieve optimal oxygen levels for fish, plants, floc growth, and solid substrate suspension (Crab *et al*., 2012).

Sampling

Water physical-chemical parameters

Water quality parameters were measured according to the standard methods of the American Public Health Association (APHA, 1989). The following parameters were measured in situ daily using a YSI 540 dissolved oxygen (DO) and Multi-functional water quality tester EZ-9909: dissolved oxygen (DO), temperature, pH, electrical conductivity, and total dissolved solids, respectively. Water nutrients samples were collected weekly for the measurement of the following nutrients: ammonia, nitrite, nitrate, and soluble phosphorus using an optical photometer YSI 9500 (YSI Incorporated, Yellow Springs, OH, USA) (±1percentage precision) (YSI, I. 2014) following the methodologies described by the manufacturer.

Phytoplankton sampling, identification and enumeration

Samples of 50 ml of phytoplankton were collected weekly using a Perspex tube fitted with nylon net. All samples in each treatment were collected from 5 different locations, mixed thoroughly, and transferred to sterile plastic bottles (Thompson, 2002). The samples were filtered with 25 µm mesh nets and preserved using Lugol iodine solution. A standard inverted light microscope with a magnification of 10 x 40 (Swift, M-4000) was used to identify and count phytoplankton cells. A sub-sample of 1ml from each sample was placed on a Sedgewick–Rafter (S–R) cell, which has 1000 fields of 1 mm^3. The S–R cell was left undisturbed for 2 minutes to allow the phytoplankton to settle. Individual phytoplankton cells were identified in 10 randomly chosen S-R cells. Phytoplankton identification to genus level was determined using keys by (Janse *et al*., 2006) and (Haney *et al*., 2013). Phytoplankton cell counts were recorded in ten randomly selected S-R cells. The number of phytoplankton cells was expressed as the number of natural units/cells per liter. The formula used to determine the total number of phytoplankton cells was as follows:

$$N = (P \times C \times 100) / L$$

Where N=the number of plankton cells or units per liter of original water;

P= the average number of plankton counted in 10 fields; the

C is the volume of concentrates (ml); L is the volume (L) of the pond water sample.

Data Analysis

One-way ANOVA was used after phytoplankton data transformation to test the effect of treatments on phytoplankton abundance using Minitab 19 software. We used Minitab 19 software to compute weekly means for each treatment and control group (total of six weeks) and performed repeated measure ANOVA analysis. We used repeated measure ANOVA to determine how the treatment altered the amount of nutrients and phytoplankton in water over time (the experimental period). The Shannon Diversity Index (Shannon-Wiener Index) measures the diversity of species in a community. A value of H = 0 indicates that the community contains only one species (Zach, 2021). We used Shannon-wiener (H') indices to assess the diversity of phytoplankton communities in treatments and control with the PAST software.

Furthermore, a generalized linear mixed model (GLM) was used to test the effect of carbon sources on response variables SRP, NH_4^+, NO_2^-, and NO_3^- with the lme (Linear Mixed Effects) function in the Statgraphics software. The model incorporated carbon sources (treatments) as a categorical variable and time (weeks 0 to 9) as a fixed effect. We also included the interaction of treatments (Carbon sources) with time (treatments * time) to test for differences in the time changes of responses. Response variables were log-transformed where necessary to meet normality assumptions. Canonical Correspondence Analysis (CCA) was used to determine the relationship between water physiochemical parameters, carbon sources, and phytoplankton among the treatments. Finally, we used PAST software to analyze CCA.

Results

Water quality parameters in the flocponic treatments and control

Among the treatments and the control, there was a significant difference in ammonia ($F_{0.05,\ 5}$=5.71, p = 0.0001), nitrite ($F_{0.05,\ 5}$=18.02, p = 0.0001), nitrate ($F_{0.05,\ 5}$=11.87, p = 0.0001), and soluble

reactive phosphorus (SRP) ($F_{0.05,\ 5}$=7.96, p = 0.0001) (Table 2). The ammonia and nitrite levels in treatments and control varied between 0.01 and 0.48 mgL^{-1}. Lucerne-hay had the highest nitrate and soluble reactive phosphorus levels, followed by Rhodes-hay, wheat-bran, maize-cob, and maize-stables. The control had the lowest levels. Temperature, DO, and TDS were statistically similar among treatments and control (Table 2).

The water nutrient analysis for exhibited that ammonia, nitrite, nitrate, and soluble reactive phosphorus concentration increased over time in the treatments and control (Figures 2 to 5). Ammonia, nitrite, nitrate, and SRP significantly ($p<0.05$) differed across all the treatments and control over time. Ammonia levels were statistically different between treatments and control (F (45, 120) = 1.54, p = 0.034) (Figure 2). There was also a significant difference between treatments over time in the following parameters: nitrite (F (45, 120) =0.94, p = 0.028) and nitrate (F (45, 120) =5.2, p = 0.0001) (Figure 3 and 4, respectively). However, there was no significant variation in SRP levels among treatments and control (Figure 5). All nutrients increased significantly after three weeks. During the first three weeks, all nutrients were below 0.5 mgL^{-1}. There was a significant increase in all the nutrients after three weeks. During the experiment period, the control group had the highest levels of ammonia and nitrite, followed by the maize-stables, maize-cob, wheat-bran, Rhodes-hay, and lucerne-hay groups (Figures 2 and 3). However, changes were noticeable in nitrate and phosphorus from week five, where carbon sources lucerne-hay exhibited the highest nitrate and phosphorus levels among the treatments and controls (Figures 4 and 5).

Table 2. Physio-chemical water parameters ($\bar{x}$ ± SE) at different treatments (carbon sources): wheat-bran, Rhodes-hay, maize-cob, maize-stables, lucerne-hay, and control (no carbon) in flocponic system.

Parameter	Wheat-bran	Rhodes-hay	Maize-cob	Maize-stables	lucerne-hay	Control	F-value	p-value
Ammonia ($mg\ L^{-1}$)	$0.3±0.02^{a}$	$0.2±0.02^{a}$	$0.3±0.02^{a}$	$0.3±0.02^{ab}$	$0.3±0.02^{a}$	$0.4±0.03^{b}$	5.71	0.0001
Nitrite ($mg\ L^{-1}$)	$0.3±0.02^{a}$	$0.3±0.02^{a}$	$0.3±0.03^{a}$	$0.4±0.04^{ab}$	$0.3±0.01^{a}$	$0.6±0.04^{b}$	18.02	0.0001
Nitrate ($mg\ L^{-1}$)	$0.7±0.05^{a}$	$0.7±0.05^{a}$	$0.7±0.05^{a}$	$0.5±0.04^{b}$	$0.9±0.06^{c}$	$0.5±0.04^{b}$	11.87	0.0001
Phosphorus ($mg\ L^{-1}$)	$0.4±0.03^{a}$	$0.5±0.05^{a}$	$0.4±0.03^{a}$	$0.6±0.03^{a}$	$0.6±0.05^{b}$	$0.4±0.03^{a}$	7.96	0.0001
Temperature (^{o}C)	$27.9±0.15^{a}$	$27.9±0.14^{a}$	$27.8±0.14^{a}$	$27.8±0.14^{a}$	$27.7±0.19^{a}$	$27.9±0.14^{a}$	0.31	0.910
D.O ($mg\ L^{-1}$)	$5.5±0.06^{a}$	$5.6±0.05^{a}$	$5.5±0.05^{a}$	$5.5±0.05^{a}$	$5.5±0.05^{a}$	$5.5±0.05^{a}$	0.58	0.717
TDS ($mg\ L^{-1}$)	$113.0±4.20^{a}$	$101.4±3.90^{a}$	$109.6±3.65^{a}$	$103.3±2.86^{a}$	$103.4±2.94^{a}$	$104.4±3.42^{a}$	1.59	0.162
pH	$8.5±0.08^{ab}$	$8.5±0.08^{ab}$	$8.3±0.07^{a}$	$8.4±0.07^{ab}$	$8.3±0.06^{a}$	$8.7±0.10^{ab}$	3.87	0.002
Salinity ($mg\ L^{-1}$)	$0.5±0.01^{a}$	$0.5±0.01^{a}$	$0.5±0.01^{a}$	$0.5±0.01^{a}$	$0.5±0.02^{a}$	$0.5±0.01^{a}$	1.29	0.266

Note: Each value represents mean ± SE; Values with varied superscripts letters (a, b, c, d, and e) within the same row are significantly different ($p<0.05$)—abbreviations: DO, dissolved oxygen; TDS, total dissolved solids.

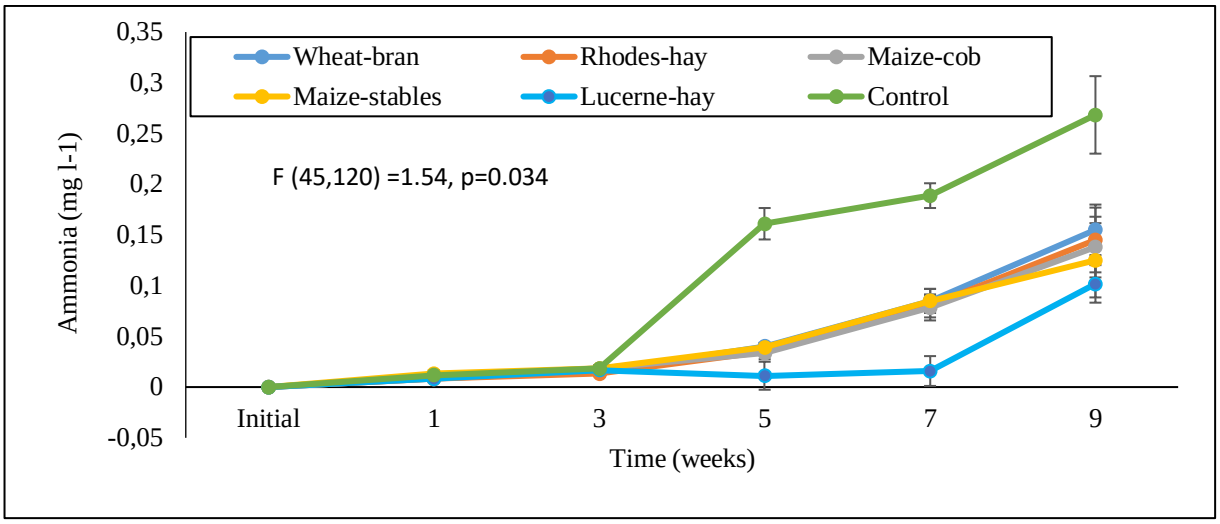

Figure 2. Variation of ammonia at different treatments (wheat-bran, Rhodes-hay, maize-cob, maize-stables, and lucerne-hay and control during the experimental period of nine weeks in the flocponic system.

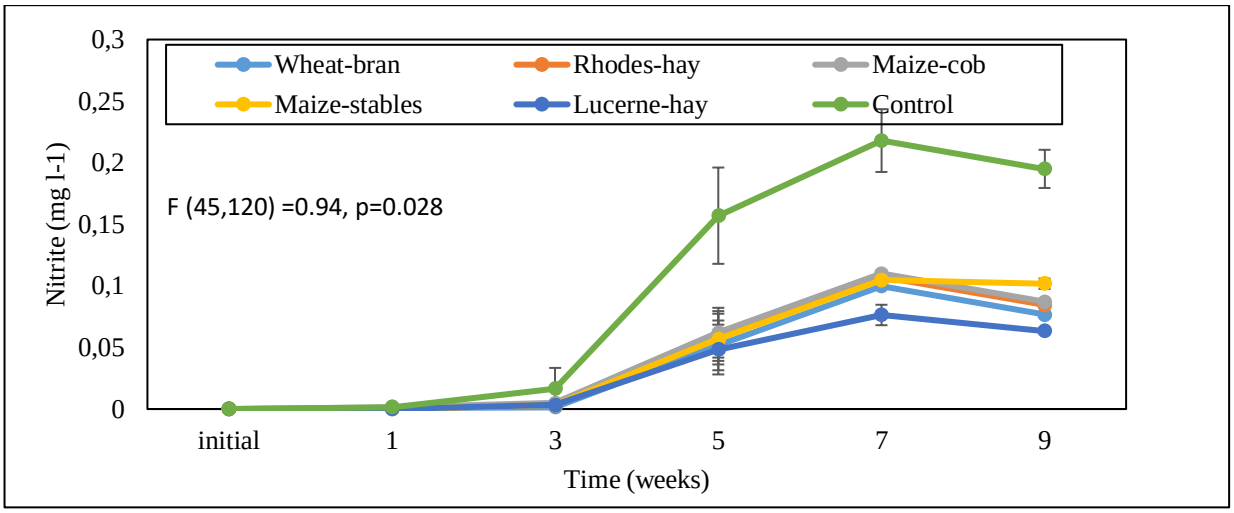

Figure 3. Variation of nitrite at different treatments (wheat-bran, Rhodes-hay, maize-cob, maize-stables, and lucerne-hay) and control during the experimental period of nine weeks in the flocponic system.

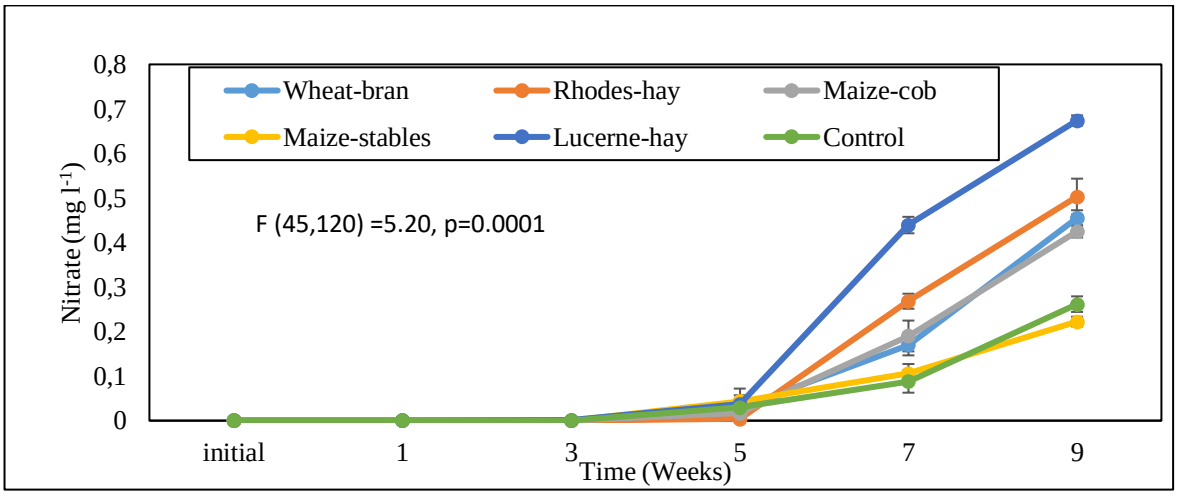

Figure 4. Variation of nitrate at different treatments (wheat-bran, Rhodes-hay, maize-cob, maize-stables, and lucerne-hay) and control during the experimental period of nine weeks in the flocponic system.

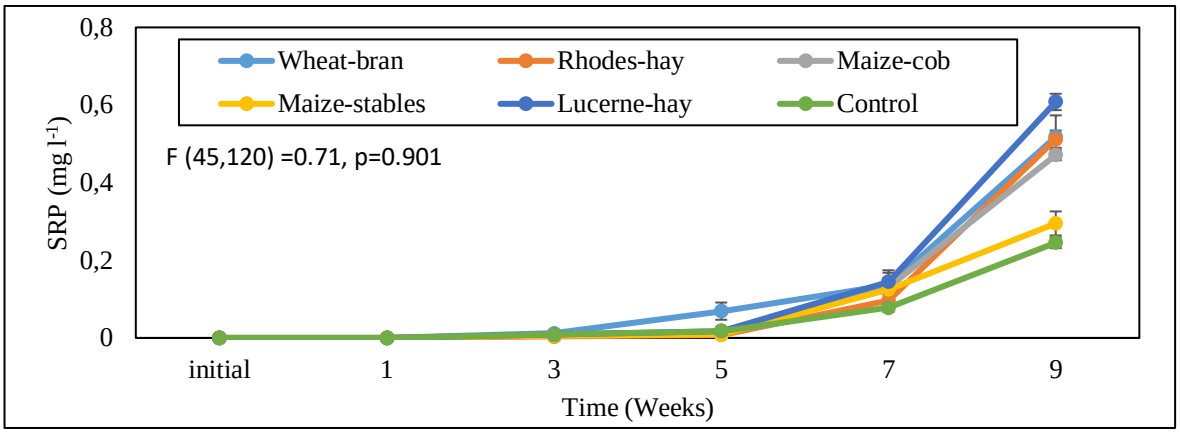

Figure 5. Variation of soluble reactive phosphorus (SRP) at different treatments (wheat-bran, Rhodes-hay, maize-cob, maize-stables, and lucerne-hay) and control during the experimental period of nine weeks in the flocponic system.

General linear mixed model (Water quality, Carbon source, and Weeks)

Ammonia, nitrite, nitrate, and phosphorus did not vary with some treatments (Table 3). However, there was a significant difference ($p<0.05$) between the lucerne-hay and maize-stable treatments on nitrate levels. The control exhibited significant differences ($p<0.05$) in all the water nutrients (ammonia, nitrite, nitrate, and soluble reactive phosphorus) (Table 3). Furthermore, there was a significant difference ($p <0.05$) in ammonia, nitrate, nitrite, and phosphorus levels over weeks. In the ANOVA table, weeks versus water nutrients were significantly different ($p<0.05$) (Table 3). Treatments versus ammonia, nitrite, and nitrate levels were statistically ($p <0.05$) different, with no significant difference in phosphorus nutrient concentration. The weeks (experimental period) * treatments significantly varied ($p <0.05$) on nitrate and ammonia water nutrient levels, while nitrite and phosphorus nutrient concentrations exhibited no significant difference (Table 3).

Table 3. Generalized mixed model for water variables at its interaction with time (weeks) and treatments (wheat-bran, Rhodes-hay, maize-cob, maize-stables, and lucerne-hay) and control

	Ammonia	Nitrite	Nitrate	Phosphorus (SRP)
Fixed effects	β(T-value) P-value	β(T-value) P-value	β(T-value) P-value	β(T-value) P-value
Wheat-bran	-0.009(-1.02)0.311	0.012(1.60)0.113	-0.006(-0.70)0.488	-0.001(-0.05)0.959
Rhodes-hay	-0.012(-1.40)0.165	0.008(1.05)0.295	-0.011(-1.37)0.173	-0.025(-1.23)0.222
Maize-cob	-0.012(-1.40)0.163	0.006(0.78)0.436	-0.002(-0.25)0.803	0.031(1.55)0.124
Maize-stables	-0.010(-1.19)0.237	0.009(1.23)0.222	-0.022(-2.68)0.008	0.027(1.37)0.174
Lucerne-hay	0.015(1.73) 0.087	0.013(1.80)0.075	0.077(9.42)0.000	-0.003(-0.15)0.884
Control	0.052(13.18)0.000	0.050(15.23)0.000	0.076(20.80)0.000	0.065(7.31)0.000
Weeks	0.013(5.64)0.00001	0.014(7.13) 0.0000	0.031(9.25) 0.00001	0.026(5.33) 0.0000
ANOVA	(F-value) p-value	(F-value) p-value	(F-value) p-value	(F-value) p-value
Weeks	(15.55)0.0001	(30.79)0.0001	(103.02)0.0001	(12.97)0.0001
Treatments	(3.35)0.007	(8.45)0.000	(19.66)0.0001	(1.34)0.254
Weeks*treatments	(1.54)0.034	(0.94)0.579	(5.20)0.0001	(0.71)0.901
R-sq (%)	65.31	75.09	91.30	56.44

Note: The 'full' model included carbon sources (treatments) wheat-bran, rhodes-hay, maize-cob, maize-stable, lucerne-hay, and control, time in weeks, treatments and treatments*time as fixed effect as explained by the model. β=coefficient

Phytoplankton in the flocponic system

Phytoplankton

The phytoplankton abundance during the experimental period in flocponic carbon-based treatments and controls is shown in Table 4. There was a significant difference ($F_{0.05, 5}$=16.30, p = 0.0001) in the Charophyta genera group abundance among the treatments and control. The lucerne-hay carbon source exhibited the highest Charophyta abundance (1.45±0.02 indsL^{-1}), and the control recorded the lowest number (1.15±0.04 indsL^{-1}). There were also significant differences in the Chlorophyta ($F_{0.05, 5}$ = 36.59, p = 0.0001) and Ochrophyta ($F_{0.05, 5}$ = 9.54, p = 0.0001) group's abundance. In the Chlorophyta and Ochrophyta groups, lucerne-hay exhibited the highest abundance, while control recorded the lowest (Table 4).

Table 5 shows the phytoplankton genera identified and the diversity at different treatments and controls. *Fragilaria, Pediastrum* (Ochrophyta), *Chlorella, Cladophora, Protococcus, Spirogyra, Spirotaenia, Volvox* (Chlorophyta), *Cosmarium, Mougeotia, Penium, Zygnema, Closterium, Desmidium, and Coleastrum* (Charophyta) are identified phytoplankton genera. Genera phytoplankton *Fragilaria, Protococcus,* and *Zygnema* genera were present in all the treatments. Furthermore, the carbon source, lucerne-hay, recorded all 13 genera of phytoplankton groups, except Pediastrum and Coleastrum, whereas the control only recorded four genera: *Fragilaria, Protococcus, Mougeotia*, and *Zygnema*. All the carbon source treatments had the highest phytoplankton diversity compared to the control. The lucerne-hay carbon source (2.48) had the most diversity, while the control (1.37) had the least (Table 5).

Figures 6-8 illustrate the dynamics of phytoplankton abundance over time. Overall, adding carbon sources increased phytoplankton abundance over time in treatments and the control. Results indicated that Ochrophyta, Chlorophyta, and Charophyta over time were not significantly (p > 0.05) different between the treatments and control. However, the post hoc test revealed variation in pattern of phytoplankton abundance over time, with some carbon sources differing from the control and other carbon source treatments. The abundance of phytoplankton in each treatment increased and stabilized starting in week 3. From week 1 to week 3, the abundance of Charophyta, Ochrophyta, and Chlorophyta rose across all treatments and control. Figure 6 displays the Charophyta abundance throughout the experimental period. Charophyta abundance significantly changed in a time-dependent manner over the study period. Week 3 exhibited the highest peak of Charophyta abundance, with 1.5 indsL^{-1} for the lucerne-hay and 1.18 indsL^{-1} for the control. Figures 7 and 8 showed comparable trends in the abundance of Chlorophyta and Ochrophyta. The highest peak of Chlorophyta and Ochrophyta abundance was detectable in week 3, and the lucerne-hay carbon source had the highest Chlorophyta (1.54 indsL^{-1}) and Ochrophyta (1.55 indsL^{-1}) peak, while the control had the lowest (Figures 7 and 8).

Table 4. Phytoplankton abundance (log10(x+1) ($\bar{x}$ ± SE) at different treatments (wheat-bran, Rhodes-hay, maize-cob, maize-stables, and lucerne-hay) and control in the flocponic experiment.

Phytoplankton	Wheat-bran	Rhodes-hay	Maize-cob	Maize-stables	Lucerne-hay	Control	F-value	p-value
Charophyta (indsL^{-1})	1.34±0.02^{a}	1.38±0.02^{a}	1.40±0.02^{a}	1.21±0.05^{b}	1.45±0.02^{c}	1.15±0.04^{b}	16.30	0.0001
Chlorophyta (indsL^{-1})	1.46±0.013^{a}	1.49±0.016^{a}	1.33±0.034^{b}	1.26±0.02^{b}	1.60±0.02^{c}	1.34±0.02^{b}	36.59	0.0001
Ochrophyta (indsL^{-1})	1.41±0.03^{a}	1.56±0.02^{a}	1.32±0.04^{b}	1.39±0.02^{c}	1.64±0.03^{d}	1.30±0.04^{e}	9.54	0.0001

Note: Each value represents mean ± SE; Values with varied superscript (a, b, c, d, e) within the same row are significantly different (p<0.05) and indsL^{-1}= individuals per litre.

Table 5. Phytoplankton diversity and abundance at different treatments (wheat-bran, Rhodes-hay, maize-cob, maize-stables, and lucerne-hay) and control in the flocponic experiment. Note: √ (present); × (absent).

Phytoplankton	Wheat-bran	Rhodes-hay	Maize-cob	Maize-stables	Lucerne-hay	Control
Ochrophyta						
Fragilaria	√	√	√	√	√	√
Pediastrum	×	×	√	×	×	×
Chlorophyta						
Chlorella	√	√	×	√	√	×
Cladophora	√	√	×	×	√	×
Protococcus	√	√	√	√	√	√
Spirogyra	√	√	×	×	√	×
Spirotaenia	√	√	×	√	√	×
Volvox	√	√	√	×	√	×
Charophyta						
Cosmarium	√	√	×	×	√	×
Mougeotia	√	√	×	×	√	√
Penium	√	√	√	√	√	×
Zygnema	√	√	√	√	√	√
Closterium	×	√	×	×	√	×
Desmidium	×	√	√	×	√	×
Coleastrum	×	×	√	×	×	×
Taxa_S	11	13	8	6	13	4
Dominance_ D	0.097	0.115	0.143	0.172	0.091	0.257
Shannon_H	2.363	2.304	2.007	1.776	2.475	1.373

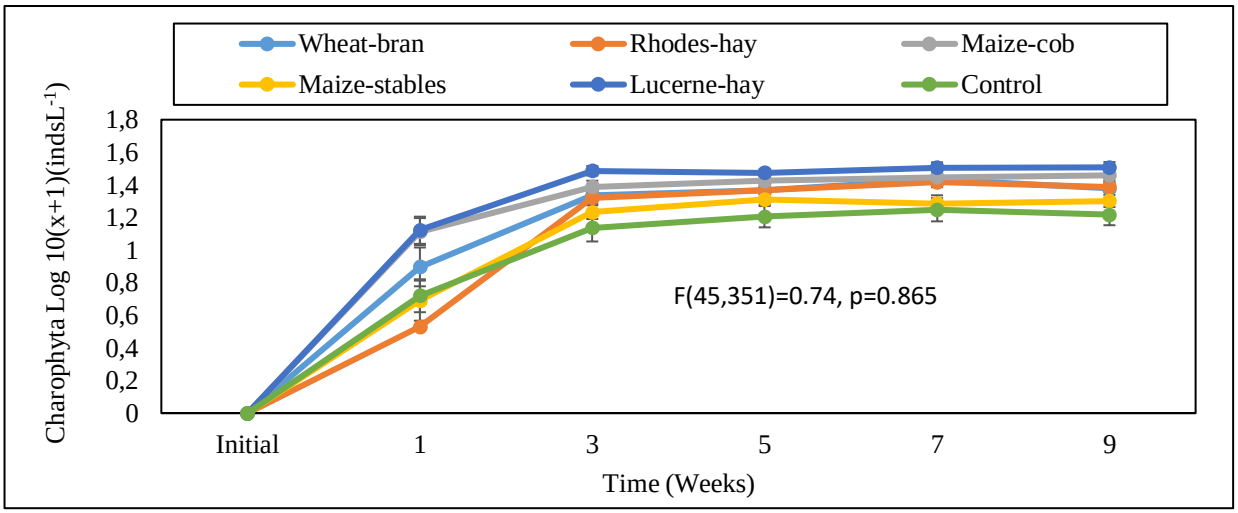

Figure 6. Variation of Charophyta in the flocponic experiment at different treatments (carbon sources) (wheat-bran, Rhodes-hay, maize-cob, maize-stables, and lucerne-hay) and control.

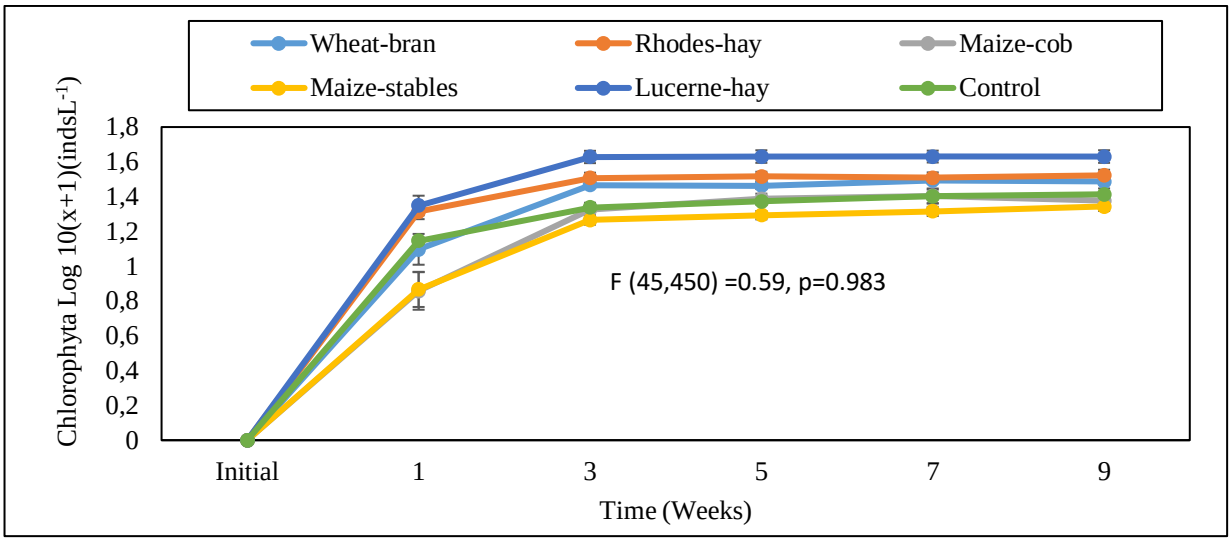

Figure 7. Variation of Chlorophyta in the flocponic experiment at different (carbon sources) treatments (wheat-bran, Rhodes-hay, maize-cob, maize-stables, and lucerne-hay) and control.

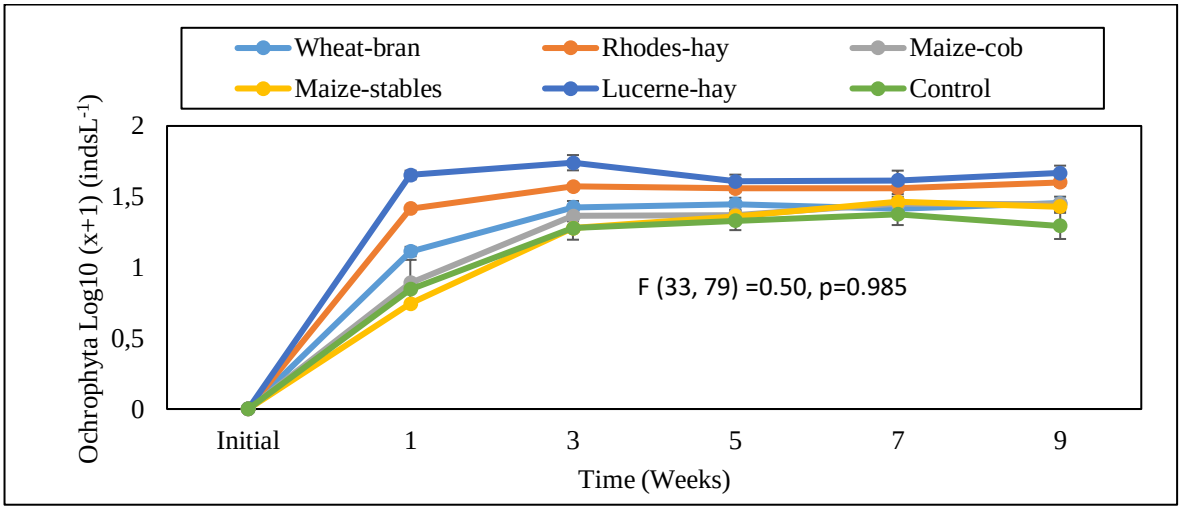

Figure 8. Variation of Ochrophyta in the flocponic experiment at different (carbon sources) treatments (wheat-bran, Rhodes-hay, maize-cob, maize-stables, and lucerne-hay) and control.

Relationship between treatments (carbon sources), phytoplankton, and water quality parameters

CCA was used to discern the possible correlations between the phytoplankton genera, the carbon sources (treatments), and the environmental variables (Figure 9). Rhodes-hay, lucerne-hay, and wheat-bran carbon sources exhibited a positive relationship with the Charophyta (*Cosmarium, Closterium,* and *Desmidium*) and Chlorophyta (*Cladophora, Spirogyra, Volvox*) phytoplankton groups, as well as nitrate and soluble reactive phosphorus environmental variables in axis 1. Along axis 2, the Charophyta (*Zygnema)*, Chlorophyta (*Chlorella, Spirotaenia*), and Ochrophyta (Fragilaria) groups had positive relationships with maize-stable, as well as electrical conductivity, temperature, ammonia, and nitrite. Charophyta (*Penium*) was positively associated with TDS, salinity, and maize-cob carbon sources. Furthermore, the control with no carbon source positively correlated with Chlorophyta (*Protococcus*) and Charophyta (*Mougeotia*) phytoplankton groups and dissolved oxygen. Generally, Rhodes-hay, lucerne-hay, and wheat-bran carbon sources with soluble reactive phosphorus (SRP) and nitrate were positively associated with the Charophyta (*Cosmarium, Closterium*, and *Desmidium*) and Chlorophyta (*Cladophora, Spirogyra,* and *Volvox*) phytoplankton groups. Maize-stable carbon sources with electrical conductivity, temperature, ammonia, and nitrite positively affected Charophyta (*Zygnema*), Chlorophyta (*Chlorella,*

Spirotaenia), and Ochrophyta (*Fragilaria*) phytoplankton. The maize-hay treatment with TDS and salinity provided good conditions only for the Charophyta *(Penium)* phytoplankton. Control with dissolved oxygen had a positive relationship with the Chlorophyta (*Protococcus*) and Charophyta (*Mougeotia*) groups of phytoplankton in a good way (Figure 9).

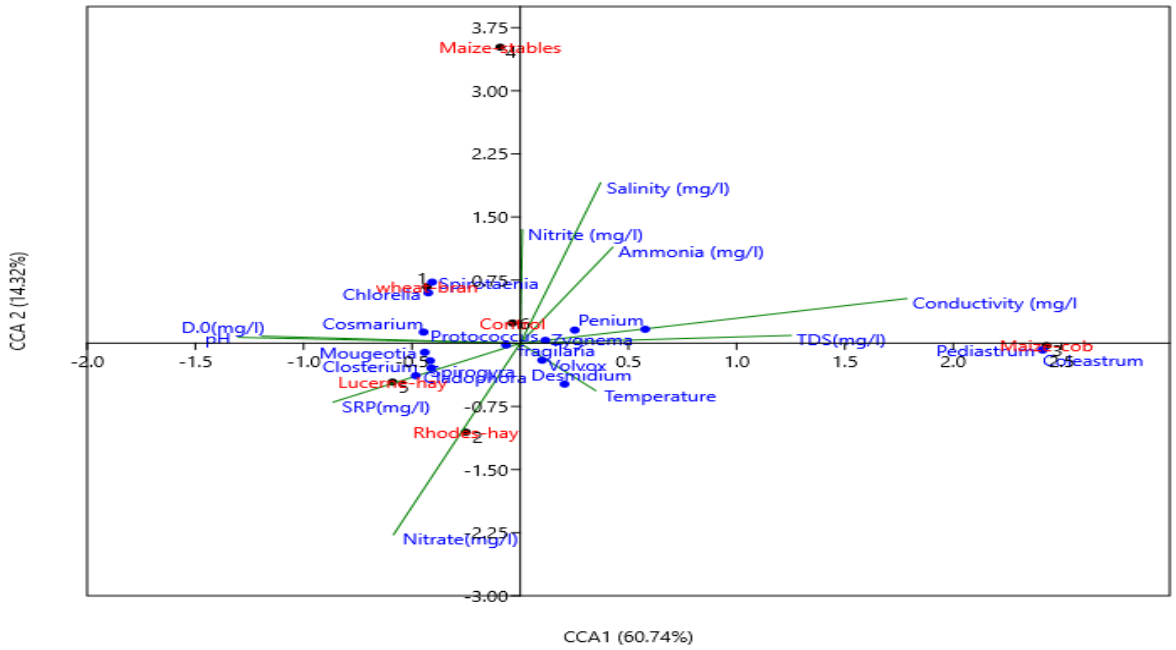

Figure 9. Triplot CCA relationships between treatments (wheat-bran, Rhodes-hay, maize-cob, and maize-stables carbon sources), control environmental variables, and phytoplankton groups.

Discussion

Water quality parameters

The temperature, salinity, and dissolved oxygen (DO) were consistent across the treatments and control throughout the study. Our results concur with Roy *et al*. (2010), Naik and Reddy (2020), Mansour *et al.* (2022), and Sharawy *et al*. (2022) findings on the farming of *L. vannamei* in biofloc systems. Hassan *et al*. (2022) found the same results for temperature (24-28ºC), pH (6.4-8.6), and DO (4.5 mg/l) when using sugarcane bagasse, rice bran, and rice straw as carbon sources in a biofloc system to grow *Litopenaeus vannamei* post-larvae. The current study also revealed slight differences in the dissolved oxygen (DO) levels between flocponic treatments with and without carbon sources, potentially due to the constant aeration of the flocponic system. Furthermore, the temperature recorded in this study was within the ideal range for biofloc and hydroponic production (Hostins *et al*., 2015; Deswati *et al*., 2021; Khanjani *et al*., 2021). During the experimental period, the consistent temperature in the greenhouse could have led to this phenomenon.

The lower pH in the treatments, unlike in the control, could be attributed to the higher carbon dioxide concentration from the respiration of microorganisms in flocponic treatments with carbon sources. The floc biomass could also consume oxygen and release carbon dioxide, leading to low pH due to the synthesis of carbonic acid. Xu *et al*. (2016) found that the carbon dioxide levels in the carbon-based biofloc treatments, originating from heterotrophic organism respirations, likely cause the dynamic changes in pH in the biofloc system. The current result corroborates Solima and Mohsen's (2022) findings that carbon treatments lower the pH levels in biofloc-based ponds. However, the current study was conducted in a flocponic system, but the findings could be similar since flocponic integrates the concept of biofloc technology.

Fish excrete total ammonia nitrogen via feces, urine, uneaten feed, the decomposition of debris, and plankton. During the experimental period, ammonia levels (0.01 to 0.03 mg/l) were within the ranges required for culturing Nile tilapia species. The ammonia levels in the carbon-based

treatments were lower than in the control. The dynamic changes in ammonia were found in treatments and control over time. The reduced ammonia levels in the flocponic treatments are likely attributed to microorganisms, such as ammonia-oxidizing bacteria, which utilize carbon as an energy source to transform ammonia into nitrite and proteins and facilitate the decomposition of organic matter. Correia *et al*. (2014) and Khanjani *et al*. (2021) indicated that ammonia and nitrite-oxidizing bacteria reduced NH_3 and nitrite in the biofloc carbon-based system compared to the control pond unit. Deng *et al.* (2018) and Soliman and Mohsen (2022) reported that the organic carbon in a biofloc technology-based system increased the number and diversity of microbial communities, particularly ammonia-oxidizing bacteria, reducing the ammonia concentration.

Furthermore, flocponics with carbon sources detected changes in ammonia over time. The lucerne-hay carbon source had the lowest ammonia level compared to other carbon sources. The solubility and composition of the carbon sources, which offer varying energy levels and surface areas necessary for bacterial development, could potentially explain the anomaly. Therefore, both the number and variety of microbes increase, promoting the process of dynamic ammonia conversion. However, there is a scarcity of investigations conducted specifically in the flocponic system. The addition of a carbon source in the biofloc system resulted in a significant increase in the growth of heterotrophic bacteria, thereby preventing the rise of ammonia levels (Deswati *et al*., 2021; Hassan *et al*., 2022; Soliman & Mohsen, 2022). Khanjani *et al*. (2021) also found that NH_3 levels decreased more when using simple carbohydrates such as molasses in a biofloc system. The faster reduction of ammonia using simple carbon sources is probably due to the better absorption and degradation of carbon as a substrate for heterotrophic bacteria that metabolize ammonia, thus improving water quality (Khanjani *et al*., 2021).

Nitrite is a vital water pollutant owing to its high toxicity (Pérez-Rostro *et al*., 2014). The primary harmful effects of NO_2 directly affect oxygen transport, the oxidation of essential chemicals, and tissue destruction (Crab *et al*., 2012). Our results revealed lower nitrite levels in flocponic treatments with carbon sources compared to the control, and this could be attributed to the bacteria's efficient conversion of ammonia and the rapid pace of nitrification. Ebeling *et al.* (2006) reported that the primary factor responsible for reducing NO_2-N levels in biofloc systems is the conversion of ammonia by bacteria within the culture unit, which can also happen in flocponic systems of the present study. Hassan *et al*. (2022) showed similar nitrite levels on the rice bran and rice straw on *Litopenaeus vannamei* post-larvae in the biofloc system. However, different carbon treatments recorded different nitrite levels; the lucerne-hay exhibited low levels, possibly due to organic carbon's absorption and degradation efficiency as a substrate for a microorganism that fastens the nitrification process.

Nitrate results from the nitrification process, and while it is one of the less hazardous inorganic nitrogen compounds, it can become a concern if its levels become too high and buildup (Mallasen & Valenti, 2006). In addition, nitrate boosts plankton production and growth (Middelburg & Nieuwenhuize, 2000). Thus, nitrate was significantly higher in the treatments compared with the control. Lucerne-hay exhibited a higher nitrate concentration but was within the acceptable range for Nile tilapia culture. Bacteria in flocponic treatments could have contributed to dynamic changes in nitrate levels compared to the control. These bacteria could have also facilitated successive ammonia oxidation to nitrite and, subsequently, to nitrate.

Aquaculture relies on phosphorus as the primary ingredient for aquatic organisms and plankton growth (Sugiura *et al*., 2006). The treatments' soluble reactive phosphorus (SRP) levels were slightly higher than in the control. The higher SRP might mean that carbon sources have influenced soluble reactive phosphorus. Butz and Vencappell (1982), Kibria *et al*. (1997), and Kong *et al*. (2020) also believe that fish feed ingredients contain a significant phosphorus fraction in a labile form; namely, the total phosphorus in fish feed, the more water-soluble phosphorus. The lucerne-hay carbon product had the highest levels of soluble reactive phosphorus compared to other carbon products and control. The lucerne-hay carbon's nature and simple sugars could have

stimulated the growth of more microbes, thereby aiding in the mineralization and production of SRP. Further, the high number of microorganisms in treatments could have facilitated the mineralization of organic carbon, waste, and solid particles into phosphorus. Ruzzi and Aroca (2015) and Brunno and Kevin (2016) reported that microorganisms in biofloc enhance phosphorus (P) availability by mineralizing organic matter and solubilizing precipitated phosphates in the culture system. Pinho *et al.* (2017) also indicated that microorganisms and planktonic communities are essential in biofloc systems as they mineralize nutrients into various elements.

Effect of different organic carbon sources on phytoplankton diversity and Abundance in the flocponic system

In the flocponic system, flocs aggregate that grow in the system are the main drivers for various activities. The phytoplankton and zooplankton are some of the complex living organism that metabolize nitrogenous waste from fish waste, uneaten feed, and debris (Castro-Mejía *et al*., 2017). Although plankton is a component of floc aggregates in biofloc systems, no published studies have examined their dynamic nature in flocponic setups. Generally, the planktonic community is essential in biofloc and aquaponic systems, as they mineralize nutrients and serve as natural food for the farmed fish species and other organisms (Green *et al*., 2014). The current study demonstrates that phytoplankton populations in all flocponic systems undergo temporal changes regardless of carbon source treatments and control. The characteristics of the organic carbon supply, including its type, solubility, and composition, could have influenced water's physical and chemical properties, resulting in fluctuating variations in phytoplankton populations over time. Biological conditions such as competition and predation could also have contributed to this phenomenon. The same is reported by Green *et al*. (2014) and Castro-Mejía *et al*. (2017), who stated that plankton's abundance changes in response to physical-chemical parameters and predators' effects.

During the experimental period, phytoplankton dominance in all flocponic systems consisted of Chlorophyta, Charophyta, and Ochrophyta. A higher abundance of Chlorophyta, Charophyta, and Ochrophyta corroborates Maica *et al.* (2011) and Pinho *et al*. (2017) with *O. niloticus* and *L. vannamei* species, respectively, but contrasts with results reported by Monroy-Dosta *et al.* (2013) in the culture of Nile tilapia in a biofloc system. The high levels of nitrate and soluble reactive phosphorus in the flocponic system with the lucerne-hay carbon product and its ability to break down may have elevated the diversity and abundance of phytoplankton growth over time. Sumitro (2021) and Soedibya *et al.* (2022) indicated that high N, P, and K levels stimulated phytoplankton growth in the biofloc system. Pinho *et al.* (2017) also discovered that the availability of nutrients and the greenhouse's sunlight exposure could cause high levels of Chlorophyta, Charophyta, and Ochrophyta. Emerenciano *et al.* (2013) indicated that phytoplankton grows well at high nitrogen and phosphorus concentrations. Such a dynamic driver might play similar functions in the flocponic system. The high ammonia concentration and absence of carbon in the control could have contributed to phytoplankton's low abundance and diversity. The concentration of water nutrients could have also contributed to the phenomenon. According to Schmittou and Rosati (1991) and Soedibya *et al*. (2022), a level of ammonia concentration that is more than 0.3 mg/l disturbs the absorption of nutrients by phytoplankton, hence hampered growth. Nevertheless, there is a lack of study on the effects of agricultural by-products as carbon sources on the makeup of plankton populations in flocponic systems or any other aquaculture system.

According to Canonical Correspondence Analysis (CCA), there was a close correlation between carbon sources, water quality parameters, and phytoplankton groups. These results corroborate with other studies, which indicated that the abiotic environment affects bacteria and plankton community structure in the aquatic environment (Xue *et al.,* 2021). Zhan *et al*. (2016) demonstrated that abiotic environmental factors, such as total ammonia nitrogen and total nitrate, significantly influence bacterial populations in *L. vannamei* culture in ponds. The addition of carbon to the flocponic system alters various ecological factors. For example, wheat-bran, Rhodes-hay,

maize-cob, maize-stables, and lucerne-hay carbon sources exhibited higher nitrate and phosphorus levels than the control. These dynamic changes in water nutrients and carbon source composition over time could have influenced the relationship between phytoplankton groups, environmental variables (water parameters), treatments, and the control. The CCA results indicated that carbon sources and water parameters influenced the phytoplankton groups, which differed among the five treatment types. There was a positive relationship between phytoplankton, carbon sources, and water parameters. All the carbon sources and other water nutrients, particularly nitrate, DO, nitrite, ammonia, and phosphorus, exhibited positive relationships with phytoplankton. Lucia *et al*. (2014) indicated that carbon and water nutrients are essential for controlling bacterioplankton. Our findings showed that water parameters, particularly nitrate, phosphorus, nitrite, ammonia, temperature, and carbon products in flocponic systems, are critical factors affecting phytoplankton community composition.

Conclusion/Recommendation

Water quality parameters such as ammonia, nitrite, and nitrate in the carbon-based flocponics were within the optimal range for the composition of the phytoplankton community. The abundance and diversity of phytoplankton significantly improved in carbon-based flocponics. The lucerne-hay and wheat-bran carbon products exhibited the highest diversity and abundance of phytoplankton. The lucerne-hay proved to be a superior carbon source due to the improved water quality and phytoplankton community composition in the flocponic system. The lucerne-hay carbon source might be rich in bacterial energy components crucial for water quality, culture species, and the phytoplankton community. The richness of lucerne-hay's bacterial energy components suggested a viable carbon source for flocponic systems and aquaculture practices. Further research should examine the impact of organic carbon sources on the dynamics of zooplankton composition in a flocponic system.

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Ethical approval

The author declares that this study complies with research and publication ethics. The experiment was conducted following the standard operating procedures (SOPs) of the University of Eldoret guidelines for handling animals. The standard operating procedures (SOPs) comply with the Prevention of Cruelty to Animals Act 1962, CAP 360 (Revised, 2012) of the laws of Kenya, and EU regulation (EC Directive 86/609/EEC).

Informed consent

Not available.

Conflicts of interest

There is no conflict of interests for publishing this study and the corresponding author is responsible on behalf of all authors' declaration.

Data availability

The authors declare that data are available from authors upon reasonable request

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Author contribution

Rono Kenneth: Conceptualization, methodology, Investigation, data curation, analysis, and writing. Geraldine Matolla: Conceptualization, methodology, writing, review, and editing

Julius O. Manyala: Conceptualization, methodology, data curation, analysis, review, and editing

Frank O. Masese: Conceptualization, methodology, data curation, analysis, review, and editing.

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